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TITLE: The Effect of Hypotensive Resuscitation and Fluid Type on Mortality, Bleeding, Coagulation and Dysfunction Inflammation in a Swine Grade V Liver Injury Model

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13. ABSTRACT (Maximum 200 Words)

Objectives: To determine the optimal fluid resuscitation after uncontrolled hemorrhagic shock in a Grade V liver injury model in swine. **Methods:** 1. Lung tissue from previously studied animals that underwent Grade V liver injury and resuscitation with lactated Ringer's, Hextend or fluid resuscitation, was assessed for mRNA expression of IL-6, TNF-alpha and GCSF. Neutrophil sequestration within alveolar walls was also assessed using myeloperoxidase staining. 2. Sixty-two animals were subjected to Grade V liver injury and uncontrolled hemorrhage for 30 minutes. Fifty animals were randomized to resuscitation with 250cc of normal saline, 3% hypertonic saline (HTS), 3% HTS with dextran, 7.5% HTS or 7.5% HTS with dextran. **Results:** 1. There was no difference in neutrophil sequestration in the lung between hemorrhaged groups. The group receiving no fluid resuscitation had a significantly greater inflammatory response than those that were resuscitated. There was no diference between LR and Hextend. 2. 3% dextran infusion resulted in the best resuscitation with respect to blood pressure and tissue oxygenation. **Conclusions:** 1. Resuscitation results in a dysfunctional inflammatory response not seen after injury alone. 2. 250cc of 3% HTS with dextran provides optimal resuscitation in this model.

14. SUBJECT TERMS
Uncontrolled hemorrhage, resuscitation, lactated Ringer's, normal saline, hextend, hypertonic saline, coagulopathy, swine

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Table of Contents

Cover..... 1

SF 298..... 2

Table of Contents..... 3

Introduction..... 4

Body..... 4

Key Research Accomplishments..... 11

Reportable Outcomes..... 12

Conclusions..... 13

Appendix 1 – EAST Manuscript..... 14

Appendix 2 – AAS Abstract..... 36

Appendix 3 – WTX 2004..... 37

Appendix 4 – 7.5HTS..... 38

INTRODUCTION:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. The goal of this proposal is to determine the ideal resuscitation regimen of swine undergoing a Grade V liver injury followed by 30 minutes of uncontrolled hemorrhagic shock. Fluids studied include lactated Ringer's (LR), Hextend and various concentrations of hypertonic saline. Fluids were evaluated based on their effects on mortality, metabolic changes, blood pressure, tissue oxygenation and inflammatory changes measured in the lung.

BODY:

Materials and Methods

We have previously reported the physiologic findings comparing LR to Hextend in our Grade V liver injury model. (Final report DAMD17-01-1-0693) In part 1 of this report, we will report our findings concerning inflammation in stored lung tissue. In part 2, we will describe the results of our study comparing 4 different hypertonic saline solutions.

Part 1

Uncontrolled hemorrhagic shock model

Thirty-eight Yorkshire crossbred swine with a mean weight of 35 kg underwent a 16-hour pre-operative fast except water ad libitum. Animals were pre-anesthetized with 8 mg/kg Telazol® (Fort Dodge Animal Health, Fort Dodge, IA) by intramuscular injection, intubated with a 6.5 mm to 7.5 mm oral endotracheal tube, and mechanically ventilated. Adequate anesthesia, assessed by monitoring jaw tone, was maintained with isoflurane (Abbott Laboratories, North Chicago, IL) and adjusted by the animal technician as needed. Respiratory rate was adjusted to maintain an end-tidal CO_2 and Pco_2 of 40 ± 4 mmHg, and tidal volume was set at 12 ± 2 cc/kg. After establishing anesthesia and mechanical ventilation, invasive monitoring devices were placed including an esophageal thermometer, left common carotid arterial catheter, and left external jugular venous catheter. Animal temperature was maintained at 38.0 ± 1.5°C using warmed fluids and external warming devices.

Six swine were randomized to a control arm. These swine were anesthetized and sacrificed immediately to obtain tissue for baseline data.

An additional six swine were randomized to a sham surgery arm. After establishing anesthesia and placing monitoring devices, these swine underwent laparotomy, suprapubic Foley catheterization, and splenectomy. The spleen was weighed and lactated Ringer's solution (LR), 3 cc/g spleen weight, was infused. The abdomen was closed with towel clamps. Anesthesia was continued for two hours prior to animal sacrifice and tissue harvesting. Data obtained from the sham animals served as a control for model effects including laparotomy, splenectomy, mechanical ventilation and anesthesia.

The remaining thirty animals were randomized to a no fluid arm (NF) or to one of two blinded resuscitation arms (LR, HEX). After establishing adequate anesthesia and

placing invasive monitoring devices, these animals also underwent laparotomy, suprapubic Foley catheterization, splenectomy and splenic volume replacement. Following a 15-minute stabilization period, pre-weighed laparotomy sponges were placed into the pelvis and inferior left and right pericolic gutters. Standardized grade V liver injuries were then created using a specially designed clamp. The clamp was closed twice over the central portion of the liver, producing a consistent injury pattern involving a large amount of parenchymal damage as well as laceration of one or more central hepatic veins. Figure 2 is a representative hepatic injury produced with this clamp. This technique resulted in injuries consistent with grade V injuries defined by the American Association for the Surgery of Trauma Organ Injury Scaling System. This model has been described in several prior studies.

Animals were allowed to hemorrhage for 30 minutes following injury. However, all animals frank hemorrhage stopped spontaneously before the 30-minute period ended. Active hemorrhage was collected with the pre-weighed sponges and by suction, avoiding disturbance of the liver. The abdomen was then sutured closed. Blood loss was determined by reweighing the sponges and suctioned blood and was reported as a mean for each group in ml/kg ± standard deviation (SD).

After the 30-minute hemorrhage period, animals randomized to the two resuscitation arms received either LR or HEX. Both commercially prepared fluids were unmodified. (LR, Baxter, Deerfield, IL: pH 6.0-7.5,130 mEq/L sodium, 109 mEq/L chloride, 4 mEq/L potassium, 3 mEq/L calcium, 28 mEq/L L-lactate; HEX, Abbott Laboratories, North Chicago, IL: pH 5.9, 143 mEq/L sodium, 124 mEq/L chloride, 3 mEq/L potassium, 5 mEq/L calcium, 0.9 mEq/L magnesium, 28 mEq/L L-lactate, 6% hetastarch, 990 mg/L destrose.) Fluids were infused as needed to achieve and maintain baseline blood pressure for 90 minutes. An infusion rate of 165 cc/min was chosen because this is one half the rate delivered by a Level I infuser and animals weighed approximately one half the weight of a normal adult human. At the end of the 90-minute resuscitation period, animals were sacrificed and tissues harvested.

Animals randomized to the no fluid arm were maintained under anesthetic conditions identical to resuscitated animals. No fluid animals were sacrificed and tissues harvested 120 minutes after injury.

Tissue Samples

The liver was removed and examined to insure comparable injuries between study arms. Samples of lung tissue were harvested through a left lateral thoracotomy for tissue levels of interlukin-6 (IL-6), granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor alpha (TNF-α) messenger ribonucleic acid (mRNA) and for assessment of neutrophils sequestered in alveolar walls. Lung tissues for mRNA analysis were flash frozen with liquid nitrogen and stored at -80° Celsius. Tissues for histologic analysis of neutrophil sequestration were stored in formalin.

Quantitative Reverse Transcription Polymerase Chain Reaction Analysis

Tissue levels of IL-6, G-CSF, and TNF-α mRNA were determined using quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR). Total RNA was isolated from flash-frozen tissue using a commercially available kit (RNeasy® Mini Kit; Qiagen Inc., Valencia, CA). RNA (5 ng for 18S and 500 ng for the gene of interest) was reverse-transcribed into cDNA with random hexamers using the SuperScript™ III

First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) under the following conditions: 10 minutes at 25°C, 50 minutes at 50°C, followed by reaction termination at 85°C for 5 minutes. Remaining RNA was removed with RNase H at 37°C for 20 minutes. Quantitative PCR was performed utilizing the TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The endogenous control, 18S ribosomal RNA, was amplified using the Assays-on-Demand primer/probe kit (Applied Biosystems, Foster City, CA). Genes of interest were amplified using custom primers and probes. All reactions were performed on the ABI Prism® 7900HT (Applied Biosystems, Foster City, CA) utilizing the following conditions: Stage 1) 2 minutes at 50°C, Stage 2) 10 minutes at 95°C, Stage 3) 40 cycles of 15 seconds of melting at 95°C followed by DNA synthesis for 1 minute at 60°C.

Primers and probes used for gene specific PCR amplification and quantification of swine IL-6, TNF-α, and G-CSF mRNA were derived from published swine sequences.[7-9] Primers and probes were used at concentrations of 300 nanomoles and 200 nanomoles respectively.

IL-6: forward primer: 5'-GCTGCTTCTGGTGATGGCTACT-3',

reverse primer: 5'-GGCATCACCTTTGGCATCTT-3',

probe: [6-FAM]CCTTCCCTACCCCGGAACGCCT[TAMRA] ;

TNF-α: forward primer: 5'-GGCCCAAGGACTCAGATCATC-3',

reverse primer: 5'-CGGCTTTGACATTGGCTACAA-3',

probe: [6-FAM]AACCTCAGATAAGCCCGTCGCCCA[TAMRA];

G-CSF: forward primer: 3'-CCTGCCCCAGAGCTTCCT-5',

reverse primer: 5'-AGCTCGGCGCCATCAG-3',

probe: [6-AM]CTGGATTTTCCTCACTTGCTCTAAGCACTTGA[TAMRA].

Neutrophil Sequestration Analysis

Lung tissues fixed in formalin, were processed and embedded in paraffin for neutrophil analysis. 5-micron sections were heated in citrate buffer for 30 minutes. The sections were then incubated with rabbit anti-myeloperoxidase antibody (Dako, 1:1600) for 45 minutes, followed by biotinylated anti-rabbit secondary and avidin/biotin/HRP (Vectastain kit; Vector Labs, Burlingame, CA). Staining was visualized by incubating with DAB for 10 minutes followed by hematoxylin counterstaining and cover-slipping. Two separate areas of lung were sampled for each animal. For each slide, five high-power fields were examined by light microscopy for the presence of neutrophils within the alveolar walls.

Statistical Analysis

A statistical software package for personal computers (SPSS, Windows Version 11.5, SPSS, Inc., Chicago, IL) was used to compare groups using independent-samples t-tests. Significance was defined as $P < 0.05$. Q-RT-PCR data are presented as Mean fold

increase ± Standard Error of the Mean. Sequestered neutrophil results are reported as the Mean number of neutrophils per high-power field ± Standard Error of the Mean.

Part 2

In part 2, 62 Yorkshire crossbred swine were randomized. Six animals were randomized to a control group and 6 were randomized to a sham group. The remaining 50 animals were randomized to 5 groups to include normal saline (NS), 3% hypertonic saline (3%), 3% hypertonic saline with dextran (3%D), 7.5% hypertonic saline (7.5%) and 7.5% hypertonic saline with dextran. (7.5%D) The methods in Part 2 of the study were identical to part 1 with mild exceptions. Spleen replacement fluid consisted of normal saline instead of LR. Normal saline was used because all fluids utilized in part 2 contained various concentrations of NaCl. Following the 30 minute uncontrolled hemorrhage period animals were resuscitated with a single 250cc bolus of study solution given over 10 minutes. The rate of study solution delivered was determined after using 12 developmental animals. Non-invasive tissue oxygenation monitoring was implemented using near infrared spectroscopy. (Hutchinson Technology) The near infrared spectroscopy patch was placed in the left groin and tissue oxygenation was measured continuously throughout the study. All other methods were identical to part 1.

Results

Part 1

The physiologic results from part 1 have been previously reported. Table 1 contains the cytokine expression data. Data were reported as fold increase above baseline and are presented as mean ± SEM. Data collected for control animals were designated baseline and assigned a value of 1. The HEX resuscitated animals had significantly more transcription of IL-6 mRNA than controls, shams and NF animals ($P < 0.01$). The LR resuscitated animals had increased IL-6 mRNA transcription compared to controls, shams and NF animals but levels failed to reach statistical significance ($P = 0.06$). IL-6 mRNA levels were not different between the LR and HEX resuscitated animals ($P = 0.51$).

G-CSF mRNA transcription was significantly elevated in both fluid resuscitation groups compared to controls, shams and the NF group ($P < 0.04$). There was no difference in G-CSF mRNA transcription between the LR and HEX resuscitation groups ($P = 0.14$).

TNF-α mRNA transcription was also significantly elevated in fluid resuscitated animals compared to controls, shams and no fluid animals ($P < 0.04$). Again, there was no difference in TNF-α mRNA levels between the LR and HEX resuscitated animals ($P = 0.31$). TNF-α mRNA transcription was also elevated in the NF group compared to controls and shams ($P < 0.01$).

Sequestered neutrophil data, reported as mean number of neutrophils per high-power field, are also presented in Table 1. Sham animals had significantly more sequestered neutrophils than control animals and all animals receiving the grade V liver injury had significantly more sequestered neutrophils than shams or controls. There was no difference in the number of sequestered neutrophils found in any of the injured animals.

Table 1.

	Control	Sham	NF	LR	HEX	p-value		
n	6	6	6	10	10	NF *v.* LR	NF *v.* HEX	LR *v.* HEX
PMNs	6.9 +/- 1.3	12 +/- 2	22 +/-3	21 +/- 7	21 +/-6	0.426	0.353	0.972
IL-6	1 +/-1.6	3 +/-3	1.4 +/-1	15 +/- 20	21 +/- 19	0.057	0.009	0.508
G-CSF	1 +/-1.0	2 +/-1	4 +/- 3	127 +/-163	325 +/- 366	0.040	0.021	0.143
TNF-α	1 +/-0.5	2 +/-2	10 +/-2	106 +/-127	167 +/- 136	0.039	0.005	0.315

Part 2

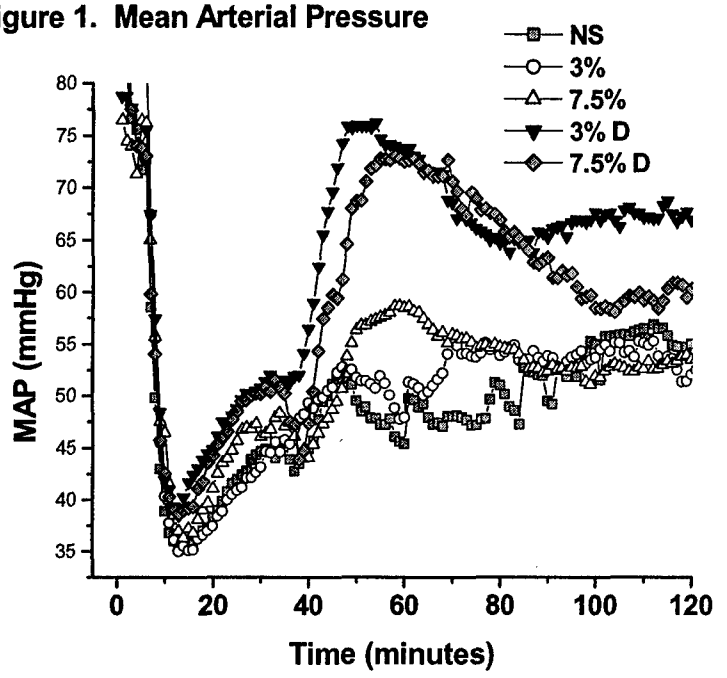
Two NS and two 3% animals did not survive to 120 minutes. All animals had similar injuries (2.1 ± 0.9 vessels). Baseline characteristics and end of study data were similar for all groups ($p>0.2$) except for urine output. Despite equal blood loss and resuscitation volumes, animals receiving 7.5% ± D produced 3 to 6 times more urine than animals receiving 3% ± D or NS, *$p<0.03$ (Table 2).

Table 2. Baseline (T0) and end of study (T120) data.

	Wt kg	T0 Temp °C	MAP T0	1° EBL ml/kg	Nadir MAP	Fluids ml/kg	UOP ml/kg	T120 Temp °C	2° EBL ml/kg
NS	33±2	37.8±0.6	82±13	23.0±7.0	33±11	7.5±0.5	1.8±2.1	38.0±0.5	1.5±0.7
3%	35±3	37.6±0.5	81±19	23.0±6.6	32±8	7.2±0.8	1.7±1.6	38.0±0.6	1.5±1.1
3%D	34±3	37.9±0.5	81±20	20.8±5.4	34±8	7.3±0.7	2.3±1.1	37.9±0.4	2.0±1.1
7.5%	34±3	37.7±0.5	76±11	21.5±5.4	32±8	7.2±0.8	6.1±4.6*	37.9±0.6	1.6±0.6
7.5%D	34±4	37.7±0.7	79±13	19.3±4.5	35±9	7.6±0.7	6.9±3.6*	37.8±0.5	2.3±0.8

Continuous MAP and StO2 data are presented in Figures 1 and 2. Injuries were created at time zero (T0). All animals experienced a precipitous drop in MAP to similar nadirs followed by a period of auto-resuscitation. The single fluid bolus was administered over 10 minutes beginning 30 minutes after injury. 7.5% saline solutions caused a brief drop in the MAP, more pronounced in the group also

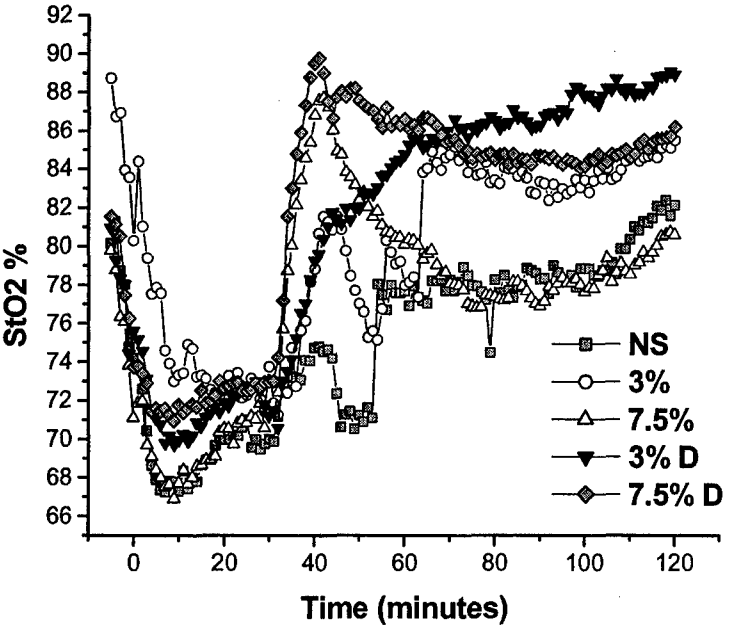
Figure 1. Mean Arterial Pressure



receiving dextran. Both HTS solutions containing dextran produced a significantly greater overall increase in MAP. The 3%D group trended toward a higher MAP at 120 minutes than the 7.5%D group.

After injury, a precipitous drop occurred in tissue oxygen saturation, mirroring the

Figure 2. Tissue Oxygen Saturation



drop in MAP. All animals reached similar StO2 nadirs followed by a period of auto-resuscitation. The 4 groups receiving HTS began improving StO2

immediately with fluid administration. 7.5% ± D solutions produced a significantly greater initial increase in StO2. However, this effect began declining within 5 minutes of completing the fluid bolus. The decline was more rapid in the 7.5% group. On the contrary, 3%D continued to improve StO2 over the 90-minute resuscitation period.

Baseline laboratory results were similar for all groups. At 120 minutes, platelet count (299±117), PTT (15.5±2.2), PT (14.2±1.3), pH (7.39±0.05), pCO_2 (48±4), pO_2 (481±99), serum lactate (2.4±1.8), and base excess (5.1±3.8) remained similar for all groups. Laboratory data showing significant differences between groups at 120 minutes is listed in Table 3. Groups receiving HTS developed hypernatremia with Na levels peaking 30 minutes after fluid infusion. Serum Na levels remained significantly different at T120. HTS groups also developed significant hyperchloremia. The degree of hypernatremia and hyperchloremia correlated with the infused fluid's NaCl concentration.

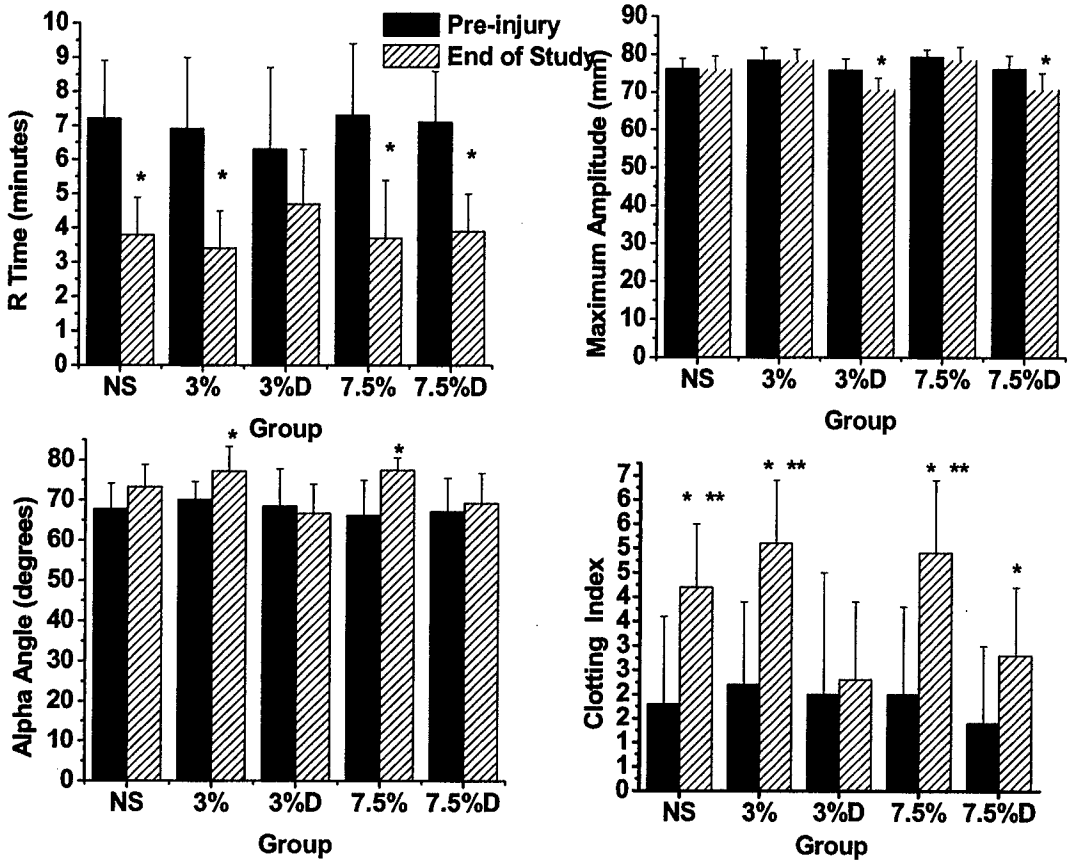
Table 3. End of study (T120) laboratory data.

	Na	Cl	Hct	Fibrinogen	Ur Na
NS	136±1.8	107±3 [b,d,e]	23.8±3.0 [b,d,e]	161±47 [b]	144±3 [b,e]
3%	139±1 [a,b,e]	109±4 [b,e]	22.4±2.7 [b,d,e]	186±54 [b]	147±3 [b,e]
3%D	140±1 [a,b,e]	111±3 [a,b,e]	19.6±2.1 [a,b,c]	150±47	146±3 [b,e]
7.5%	147±1 [a,c,d]	118±5 [a,c,d]	20.7±2.2 [a,b]	185±51 [b]	153±5 [a,c,d]
7.5%D	148±1 [a,c,d]	120±4 [a,c,d]	16.7±2.7 [a,c,d,e]	127±31 [c,e]	154±3 [a,c,d]
$p<0.05$ a *vs.* NS, b *vs.* 7.5%D, c *vs.* 3%, d *sv.* 3%D, e *vs.* 7.5%					

Significant anemia and relative hypofibrinogenemia developed in HTS groups, and was exacerbated by the addition of dextran. HTS groups developed elevated urine Na levels corresponding to serum Na.

TEG data are shown in Figure 3. Reaction (R) time represents the time to onset of clot formation. A significant decrease in R time occurred in all groups except 3%D. The alpha angle represents the rapidity of fibrin buildup and cross-linking. The alpha angle did not increase in animals receiving dextran. Maximum amplitude (MA) is a measurement of clot strength and is affected by platelet number and function as well as by fibrinogen level. The MA decreased significantly in animals receiving dextran. Clotting index (CI) is a calculated measurement of overall coagulation function derived from all measured values. CI increases significantly in all animals except those receiving 3%D

Figure 3. Thrombelastography Data. p<0.05 *v. Pre-injury, **v. 3%D, 7.5%D



Cytokine analysis Part 2

mRNA gene expression measured in the lung is shown in Table 4.

Table 4.

Cohort	GCSF	IL-6	TNF-a
Control	1.00 ±2.72 [a]	1.00 ±1.32	1.00 ±0.95
Sham	1.39 ±3.65[a]	2.36 ±4.37	2.19 ±1.70
NS	1.80 ±4.24[b]	12.57 ±16.51	4.42 ±7.46
3S	1.31 ±3.06	6.37 ±9.13	1.61 ±2.74
3D	0.76 ±1.72	8.28 ±11.72	1.01 ±1.42[c]
7.5S	0.66 ±1.50[a]	3.92 ±5.59	0.80 ±1.14
7.5D	2.50 ±5.55	30.17 ±58.10	2.11 ±3.89

[a] p<0.05 versus 7.5D, [b] p<0.05 versus 7.5S, [c] p<0.05 versus sham

As shown in Table 4, resuscitation with 7.5%D results in increased expression of GCSF. This effect is not seen when dextran is not added to 7.5% HTS. Resuscitation with 3%D results in decreased TNF-alpha expression compared to sham animals and equivalent TNF-alpha expression compared to control animals.

KEY RESEARCH ACCOMPLISHMENTS:

1. Aggressive resuscitation following uncontrolled hemorrhagic shock results in dysfunctional inflammation measured in lung parenchyma. The effect is

equivalent with lactated Ringer's resuscitation and Hextend resuscitation. This effect is not seen when animals are not resuscitated.

2. Injury and uncontrolled hemorrhagic shock results in increased alveolar neutrophils. This effect is equivalent in animals given no fluid, lactated Ringer's and Hextend.
3. The addition of dextran to hypertonic saline resuscitation solutions results in a more rapid increase in blood pressure following uncontrolled hemorrhagic shock.
4. 7.5% hypertonic saline solutions with and without dextran produce a more rapid elevation in tissue oxygenation than 3% solutions or normal saline.
5. A single bolus of 3% hypertonic saline results in the most persistent elevations of blood pressure and tissue oxygenation over 2 hours compared to other hypertonic saline solutions.
6. Significant hypercoagulability occurred in all animals except for those animals resuscitated with 3% dextran.
7. Resuscitation with 7.5%D results in increased GCSF mRNA expression in the lungs.
8. Following uncontrolled hemorrhagic shock, blood pressure spontaneously rises suggesting that autoresuscitation occurs. This rise in blood pressure correlates with an increase in tissue oxygenation.

REPORTABLE OUTCOMES

Part 1 of this work was presented at the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. It is also scheduled for presentation at the Eastern Association for the Surgery of Trauma in January 2005. Appendix 1 is the manuscript submitted to the Journal of Trauma. The abstract was also published in the Journal of Trauma:

Watters JM, Jackson T, Muller PJ, Malinoski D, Todd SR, **Schreiber MA**. Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury. Journal of Trauma. 2004;57:1378.

The spontaneous increase in blood pressure and tissue oxygenation that occurs after hemorrhagic shock was presented at the 2004 meeting of the Association of Academic Surgeons. The abstract was published in the Journal of Surgical Research:

Differding JA, Watters JM, Muller PJ, **Schreiber MA**. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. Journal of Surgical Research. 2004;126:336-337. (Appendix 2)

A manuscript will also be submitted to the Journal of Surgical Research.

The physiologic results of resuscitation with hypertonic saline solutions in Part 2 was the winner of the basic science portion of the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. This paper is also accepted for presentation at the Western Trauma Association in February of 2005.

The abstract (Appendix 3) will be published in the Journal of Trauma and a manuscript will also be submitted to the Journal of trauma. The cytokine expression results following resuscitation with hypertonic saline solutions was also presented at the Northwest Region of the American College of Surgeons Resident's trauma paper competition in December 2004. This paper is also accepted for presentation at the Society of University Surgeons meeting in 2005. (Abstract is Appendix 4) It will be submitted to the journal Surgery for publication.

CONCLUSIONS:

A single bolus of 3% hypertonic saline with dextran provides optimal resuscitation in a prehospital model of uncontrolled hemorrhagic shock with respect to blood pressure, tissue oxygenation, coagulation and inflammation. Survival studies and the study of novel fluids are the next planned steps in this research process.

Title Page

Fluid Resuscitation Increases Inflammatory Response to Traumatic Injury

Resuscitation Increases Inflammatory Response

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Abstract

Background: The debate continues over type and quantity of fluid to administer for resuscitation following traumatic injury. This study aimed to examine effects of resuscitation with lactated Ringer's (LR) and Hextend® (HEX) on the inflammatory response following uncontrolled hemorrhagic shock (UHS).

Methods: 38 swine were randomized. Control swine were anesthetized and sacrificed. Sham swine underwent laparotomy, splenectomy and 2 hours of anesthesia. UHS swine received a grade V liver injury after laparotomy and splenectomy and were randomized to no fluid (NF) resuscitation or to blinded resuscitation 30 minutes after injury with LR or HEX. Fluids were infused as needed to maintain baseline blood pressure for 90 minutes. Lung tissue mRNA levels of interleukin-6 (IL-6), granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor alpha (TNF-α) were determined. Lung sections were examined for neutrophils (PMNs) sequestered within alveolar walls.

Results: All UHS animals survived and initial blood loss was similar between groups. Mean arterial pressures (MAPs) were similar for all UHS animals until resuscitation was initiated. MAPs of resuscitated animals remained similar and were significantly higher than MAPs of the NF animals. Sequestered PMNs were equally elevated in all UHS animals. Cytokine analysis showed increased IL-6, G-CSF, and TNF-α gene transcription in resuscitated swine compared to no fluid swine. LR and HEX resuscitated swine tissue mRNA levels showed no differences.

Conclusions: Fluid resuscitation following solid organ injury and uncontrolled hemorrhage results in greater pro-inflammatory gene transcription than no resuscitation. LR and HEX resuscitation have equivalent effects on indices of inflammation in the lungs.

Key Words: Hextend®, Lactated Ringers, Resuscitation, Inflammation, Cytokines, Crystalloid, Colloid, Hemorrhagic shock

Text:

INTRODUCTION

Therapeutic intervention following traumatic injury invariably includes the administration of intravenous fluids. However, the debate continues over what type of fluid to use and how much to administer. Warnings about the ill effects of excess or unnecessary fluid resuscitation date back over a century. In 1911 George H. Evans cautioned, "Under certain circumstances saline solutions are productive of great harm to the tissues of the body, and are even capable of producing death."[1] He felt that sodium chloride solutions were being administered indiscriminately and that the morbidity and mortality caused by over exuberant fluid resuscitation was often incorrectly attributed to the patients' underlying conditions. A more recent and provocative study of penetrating torso trauma patients suggests that fluid administration should be delayed until operative control of bleeding is established.[2] Mortality and morbidity rates were lower in the group of patients in whom fluid resuscitation was not initiated in the field but delayed until operative intervention took place.

With this study we sought to examine two questions. First, whether crystalloid or colloid resuscitation following uncontrolled hemorrhagic shock would result in different effects on indices of inflammation in the lung, and second, whether withholding fluid resuscitation would result in up or down regulation of the inflammatory response. A previous study conducted by our group using this same model, compared the effects of normal saline (NS) and lactated Ringer's (LR) resuscitation on indices of inflammation in the lungs of swine subjected to grade V liver injuries and uncontrolled hemorrhagic shock. No difference in inflammatory response was identified at two hours after injury between the two resuscitation groups. However, LR resuscitated animals did better from a physiologic perspective. Based on our previous data, we chose LR for this study. Hextend® (HEX) was chosen for the study's colloid solution. HEX is a synthetic colloid in a balanced physiologic salt solution. We hypothesized

that fluid resuscitation would lead to increased elaboration of dysfunctional inflammation and that no difference would be found between crystalloid and colloid resuscitation.

MATERIALS AND METHODS

The study design was a prospective randomized, blinded, controlled trial. The Institutional Animal Care and Use Committee at Oregon Health & Science University approved the protocol. This facility adheres to the National Institutes of Health guidelines for the use of laboratory animals.

Uncontrolled hemorrhagic shock model

Thirty-eight Yorkshire crossbred swine with a mean weight of 35 kg underwent a 16-hour pre-operative fast except water ad libitum. Animals were pre-anesthetized with 8 mg/kg Telazol® (Fort Dodge Animal Health, Fort Dodge, IA) by intramuscular injection, intubated with a 6.5 mm to 7.5 mm oral endotracheal tube, and mechanically ventilated. Adequate anesthesia, assessed by monitoring jaw tone, was maintained with isoflurane (Abbott Laboratories, North Chicago, IL) and adjusted by the animal technician as needed. Respiratory rate was adjusted to maintain an end-tidal CO_2 and Pco_2 of 40 ± 4 mmHg, and tidal volume was set at 12 ± 2 cc/kg. After establishing anesthesia and mechanical ventilation, invasive monitoring devices were placed including an esophageal thermometer, left common carotid arterial catheter, and left external jugular venous catheter. Animal temperature was maintained at 38.0 ± 1.5°C using warmed fluids and external warming devices.

Six swine were randomized to a control arm. These swine were anesthetized and sacrificed immediately to obtain tissue for baseline data.

An additional six swine were randomized to a sham surgery arm. After establishing anesthesia and placing monitoring devices, these swine underwent laparotomy, suprapubic Foley catheterization, and splenectomy. The spleen was weighed and lactated Ringer's solution (LR), 3 cc/g spleen weight, was infused. The abdomen was closed with towel clamps. Anesthesia was continued for two

hours prior to animal sacrifice and tissue harvesting. Data obtained from the sham animals served as a control for model effects including laparotomy, splenectomy, mechanical ventilation and anesthesia.

The remaining thirty animals were randomized to a no fluid arm (NF) or to one of two blinded resuscitation arms (LR, HEX). After establishing adequate anesthesia and placing invasive monitoring devices, these animals also underwent laparotomy, suprapubic Foley catheterization, splenectomy and splenic volume replacement. Following a 15-minute stabilization period, pre-weighed laparotomy sponges were placed into the pelvis and inferior left and right pericolic gutters. Standardized grade V liver injuries were then created using a specially designed clamp, shown in Figure 1. The clamp was closed twice over the central portion of the liver, producing a consistent injury pattern involving a large amount of parenchymal damage as well as laceration of one or more central hepatic veins. Figure 2 is a representative hepatic injury produced with this clamp. This technique resulted in injuries consistent with grade V injuries defined by the American Association for the Surgery of Trauma Organ Injury Scaling System.[3] This model has been described in several prior studies.[4-6]

Animals were allowed to hemorrhage for 30 minutes following injury. However, all animals frank hemorrhage stopped spontaneously before the 30-minute period ended. Active hemorrhage was collected with the pre-weighed sponges and by suction, avoiding disturbance of the liver. The abdomen was then sutured closed. Blood loss was determined by reweighing the sponges and suctioned blood and was reported as a mean for each group in ml/kg ± standard deviation (SD).

After the 30-minute hemorrhage period, animals randomized to the two resuscitation arms received either LR or HEX. Both commercially prepared fluids were unmodified. (LR, Baxter, Deerfield, IL: pH 6.0-7.5,130 mEq/L sodium, 109 mEq/L chloride, 4 mEq/L potassium, 3 mEq/L calcium, 28 mEq/L L-lactate; HEX, Abbott Laboratories, North Chicago, IL: pH 5.9, 143 mEq/L sodium, 124 mEq/L chloride, 3 mEq/L potassium, 5 mEq/L calcium, 0.9 mEq/L magnesium, 28 mEq/L L-lactate, 6% hetastarch, 990 mg/L destrose.) Fluids were infused as needed to

achieve and maintain baseline blood pressure for 90 minutes. An infusion rate of 165 cc/min was chosen because this is one half the rate delivered by a Level I infuser and animals weighed approximately one half the weight of a normal adult human. At the end of the 90-minute resuscitation period, animals were sacrificed and tissues harvested.

Animals randomized to the no fluid arm were maintained under anesthetic conditions identical to resuscitated animals. No fluid animals were sacrificed and tissues harvested 120 minutes after injury.

Tissue Samples

The liver was removed and examined to insure comparable injuries between study arms. Samples of lung tissue were harvested through a left lateral thoracotomy for tissue levels of interlukin-6 (IL-6), granulocyte colony stimulating factor (G-CSF), and tumor necrosis factor alpha (TNF-α) messenger ribonucleic acid (mRNA) and for assessment of neutrophils sequestered in alveolar walls. Lung tissues for mRNA analysis were flash frozen with liquid nitrogen and stored at -80° Celsius. Tissues for histologic analysis of neutrophil sequestration were stored in formalin.

Quantitative Reverse Transcription Polymerase Chain Reaction Analysis

Tissue levels of IL-6, G-CSF, and TNF-α mRNA were determined using quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR). Total RNA was isolated from flash-frozen tissue using a commercially available kit (RNeasy® Mini Kit; Qiagen Inc., Valencia, CA). RNA (5 ng for 18S and 500 ng for the gene of interest) was reverse-transcribed into cDNA with random hexamers using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) under the following conditions: 10 minutes at 25°C, 50 minutes at 50°C, followed by reaction termination at 85°C for 5 minutes. Remaining RNA was removed with RNase H at 37°C for 20 minutes. Quantitative PCR was performed utilizing the TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The endogenous control, 18S ribosomal RNA, was amplified using the Assays-on-Demand primer/probe kit (Applied

Biosystems, Foster City, CA). Genes of interest were amplified using custom primers and probes. All reactions were performed on the ABI Prism® 7900HT (Applied Biosystems, Foster City, CA) utilizing the following conditions: Stage 1) 2 minutes at 50°C, Stage 2) 10 minutes at 95°C, Stage 3) 40 cycles of 15 seconds of melting at 95°C followed by DNA synthesis for 1 minute at 60°C.

Primers and probes used for gene specific PCR amplification and quantification of swine IL-6, TNF-α, and G-CSF mRNA were derived from published swine sequences.[7-9] Primers and probes were used at concentrations of 300 nanomoles and 200 nanomoles respectively.

IL-6: forward primer: 5'-GCTGCTTCTGGTGATGGCTACT-3',
reverse primer: 5'-GGCATCACCTTTGGCATCTT-3',
probe: [6-FAM]CCTTCCCTACCCCGGAACGCCT[TAMRA] ;

TNF-α: forward primer: 5'-GGCCCAAGGACTCAGATCATC-3',
reverse primer: 5'-CGGCTTTGACATTGGCTACAA-3',
probe: [6-FAM]AACCTCAGATAAGCCCGTCGCCCA[TAMRA];

G-CSF: forward primer: 3'-CCTGCCCCAGAGCTTCCT-5',
reverse primer: 5'-AGCTCGGCGCCATCAG-3',
probe: [6-AM]CTGGATTTTCCTCACTTGCTCTAAGCACTTGA[TAMRA].

Neutrophil Sequestration Analysis

Lung tissues fixed in formalin, were processed and embedded in paraffin for neutrophil analysis. 5-micron sections were heated in citrate buffer for 30 minutes. The sections were then incubated with rabbit anti-myeloperoxidase antibody (Dako, 1:1600) for 45 minutes, followed by biotinylated anti-rabbit secondary and avidin/biotin/HRP (Vectastain kit; Vector Labs, Burlingame, CA). Staining was visualized by incubating with DAB for 10 minutes followed by hematoxylin counterstaining and cover-slipping. Two separate areas of lung were sampled for each animal. For each slide, five high-power fields were examined by light microscopy for the presence of neutrophils within the alveolar walls.

Statistical Analysis

A statistical software package for personal computers (SPSS, Windows Version 11.5, SPSS, Inc., Chicago, IL) was used to compare groups using independent-samples t-tests. Significance was defined as $P < 0.05$. Q-RT-PCR data are presented as Mean fold increase ± Standard Error of the Mean. Sequestered neutrophil results are reported as the Mean number of neutrophils per high-power field ± Standard Error of the Mean.

RESULTS

All animals survived to 120 minutes and had grade V liver injuries on autopsy with an average of 1.2 central hepatic veins injured per animal. Figure 3 demonstrates composite mean arterial blood pressure (MAP) curves for the no fluid and two resuscitation arms. Time zero is the point at which the liver injury was created. All animals experienced a precipitous drop in blood pressure followed by a period of auto-resuscitation. During active fluid resuscitation, blood pressures were maintained at pre-injury baseline in the LR and HEX resuscitation arms. The mean arterial pressures of fluid resuscitated animals are significantly higher than animals receiving no fluid. However, no statistical difference exists between the MAPs of the LR and HEX animals.

Mean blood loss, resuscitation and urine output volumes are shown in Figure 4. Despite equal pre-resuscitation blood loss, LR: 22 ± 5 mL/kg; HEX: 22 ± 8 mL/kg; NF: 18 ± 6mL/kg ($P > 0.1$), swine resuscitated with LR required almost 3 times as much fluid to maintain pre-injury mean arterial pressure as animals receiving HEX (119 ± 78 ml/kg v. 40 ± 21 mL/kg, $P = 0.01$). The LR (21 ± 13 mL/kg) resuscitated animals also produced significantly more urine than the HEX (10 ± 4 mL/kg, $P = 0.03$) or NF (4 ± 2 mL/kg, $P < 0.01$) animals. HEX resuscitated animals produced significantly more urine than NF animals ($P < 0.01$).

Laboratory data collected at the end of the study are shown in Table 1. Significant differences developed between groups in serum sodium and chloride. However, these differences were due to small standard deviations and were not

clinically significant. There were no differences between groups in pH, pO_2, or pCO_2. Base excess was lower in LR animals compared to HEX animals (P = 0.04). Animals resuscitated with LR had significantly higher lactate levels than those resuscitated with HEX. There was no significant difference in lactate levels between the NF group and either resuscitation group. Figure 5 compares baseline to end of study hematocrit for each group. At the end of study, the resuscitated animals developed profound anemia compared to their baselines (P < 0.001) and compared to the NF animals (P < 0.001).

Table 2 contains the cytokine expression data. Data were reported as fold increase above baseline and are presented as mean ± SEM. Data collected for control animals were designated baseline and assigned a value of 1. The HEX resuscitated animals had significantly more transcription of IL-6 mRNA than controls, shams and NF animals (P < 0.01). The LR resuscitated animals had increased IL-6 mRNA transcription compared to controls, shams and NF animals but levels failed to reach statistical significance (P = 0.06). IL-6 mRNA levels were not different between the LR and HEX resuscitated animals (P = 0.51).

G-CSF mRNA transcription was significantly elevated in both fluid resuscitation groups compared to controls, shams and the NF group (P < 0.04). There was no difference in G-CSF mRNA transcription between the LR and HEX resuscitation groups (P = 0.14).

TNF-α mRNA transcription was also significantly elevated in fluid resuscitated animals compared to controls, shams and no fluid animals (P < 0.04). Again, there was no difference in TNF-α mRNA levels between the LR and HEX resuscitated animals (P =0.31). TNF-α mRNA transcription was also elevated in the NF group compared to controls and shams (P < 0.01).

Sequestered neutrophil data, reported as mean number of neutrophils per high-power field, are presented in Figure 6. Sham animals had significantly more sequestered neutrophils than control animals and all animals receiving the grade V liver injury had significantly more sequestered neutrophils than shams or controls. There was no difference in the number of sequestered neutrophils found in any of the injured animals.

DISCUSSION

The data from this study indicate that fluid resuscitation following uncontrolled hemorrhagic shock increases pro-inflammatory gene transcription including IL-6, G-CSF and TNF-α in lung tissue. Only TNF-α is significantly increased in un-resuscitated injured animals compared to shams and controls. LR and HEX resuscitation result in equally up-regulated transcription of these pro-inflammatory cytokines.

Neutrophil sequestration in the lungs is equally increased in all traumatically injured animals independent of resuscitation. The increased neutrophil sequestration in the sham group represents the effects of our model including 120 minutes of mechanical ventilation and anesthesia and laparotomy with splenectomy. Solid organ injury and uncontrolled hemorrhage significantly increase the number of sequestered neutrophils independent of resuscitation.

The host immune response to traumatic injury is extremely complex and may vary based on the nature of the injury sustained. As a result, successful modulation of this response may prove difficult. However, understanding the influence of fluid resuscitation on the inflammatory response is critical for exploring potential avenues of modulation.

TNF-α is elaborated primarily by activated macrophages. The local production of TNF-α by pulmonary macrophages plays an early and important role in the induction of endothelial chemotactic and activating factors for mononuclear phagocytes.[10-12] Bronchoalveolar lavage fluid from patients with acute respiratory distress syndrome (ARDS) contains elevated levels of TNF-α compared to serum.[13] Elevation of TNF-α transcription in all injured animals indicates that UHS from hepatic injury leads to up-regulation of this pro-inflammatory cytokine in the lung. The signaling pathway leading to elaboration of TNF-α in the lung following UHS warrants further investigation. Local up-regulation of TNF-α in the lung may account for the equally increased numbers of sequestered neutrophils seen in the UHS animals. Although fluid resuscitation leads to further significant increases TNF-α transcription, it does not lead to

significantly increased numbers of sequestered neutrophils. What is not known is whether the excess TNF-α transcription following fluid resuscitation leads to increased neutrophil priming, placing these animals at increased risk for neutrophil-mediated endothelial injury or at increased risk of later ARDS. It is also unknown if excess local TNF-α transcription contributes to exaggerated systemic TNF-α release and subsequent multiorgan injury.

While TNF-α is primarily a pro-inflammatory cytokine, IL-6 and G-CSF have complex pro- and anti-inflammatory properties. Despite its duel roles, elevated IL-6 production has been associated with poor outcome in numerous studies.[14-16] In a rat model of traumatic hemorrhage, IL-6 was found to play an important role in post-trauamtic hepatic dysfunction.[17] IL-6 also induces acute phase protein synthesis in hepatocytes.[18] G-CSF stimulates the proliferation and differentiation of precursor cells committed to the neutrophil cell type. G-CSF can also enhance the survival and activate the immunological functions of mature neutrophils. Additionally, G-CSF reduces the amount of pro-inflammatory cytokines released by activated monocytes and macrophages, thus preventing over-activation of the immune system.[19] Intratracheal injection of G-CSF in rats leads to increased interstitial and alveolar neutrophils and marked pulmonary edema.[20] In this model, UHS alone is insufficient to lead to increases in IL-6 and G-CSF transcription. Following fluid resuscitation, both IL-6 and G-CSF transcription are significantly increased. This increased transcription may be induced by factors released from distal sites during reperfusion or by direct effects of the fluids on lung tissues.

In our model of UHS, LR and HEX resuscitation have equivalent effects on indices of inflammation in the lungs. This finding adds to previous in vitro work demonstrating a similar dose responsive increase in neutrophil activation and adhesion by both LR and hydroxyethyl starch solution 6%. [21] Another study examining endothelial cell E-selectin expression demonstrated no increase after exposure to hetastarch and TNF-α stimulation. [22] Volume therapy with hydroxyethyl starch solution has been associated with superior improvements in hemodynamics and a reduction in pulmonary edema.[23,24] Restoration and

maintenance of pre-injury mean arterial pressure was achieved with significantly less HEX compared to LR in our resuscitated animals. The use of HEX for resuscitation did not increase dysfunctional inflammation in our model and may be beneficial in limiting total fluid volume infused. The time line of our model needs to be extended to determine if the use of colloid effects mortality or late morbidity.

Advantages and limitations

This animal model of uncontrolled hemorrhagic shock from solid organ injury offers the distinct advantage of a consistent injury pattern and similar blood loss across subjects. Attempting a similar study in humans would be fraught with difficulty in matching subjects for injury pattern and degree of shock.

However, this study has several limitations. It is conducted in anesthetized swine rather than in non-anesthetized humans. Anesthesia is known to influence catecholamine response and invariably influences the animals' hemodynamic responses to injury. The sham group controls for these effects and allows us to draw conclusions about the effects of injury and resuscitation on the indices of inflammation in the lung. Altering the model to eliminate inhaled anesthesia and mechanical ventilation would certainly improve its clinical relevance.

Mechanical ventilation abrogates hypoxia developing. Hypoxia has been shown to induce the rapid expression of IL-8 from pulmonary macrophages, and is associated with an increased incidence of ARDS.[25] Hypoxia also increases alveolar macrophage release of TNF-α.[26] Cultured human keratinocytes increase expression of G-CSF under hypoxic conditions.[27] Prevention of hypoxia through mechanical ventilation may significantly effect the up-regulation of cytokine transcription.

Cost is a deterrent to large sample size using a swine model and due to intra-animal variations and small sample size type 2 statistical errors may occur. In addition to cost, the entire swine genome has not been sequenced limiting the number of inflammatory mediators we can examine. Similarly, investigating circulating and local protein products are limited by available reagents.

CONCLUSION

Solid organ injury and uncontrolled hemorrhagic shock lead to sequestration of neutrophils in alveolar walls. This process may be mediated by early elaboration of TNF-α. The administration of crystalloid (LR) or colloid (Hextend®) after 30 minutes of shock induces significant local up-regulation of IL-6, and G-CSF, and further up-regulation of TNF-α gene transcription. The increase in pro-inflammatory cytokine mRNA does not occur in the absence of fluid resuscitation. Additional studies to more fully describe the effects of fluid resuscitation on the inflammatory response to traumatic injury are needed if we hope to modulate the response to our patients' benefits.

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FIGURE LEGENDS

Figure 1. **Liver injury clamp.** This specially designed clamp is closed over the central portion of the liver.

Figure 2. **Grade V hepatic injury.** Representative injury created by the specially designed clamp in figure 1. A large amount of parenchymal destruction is accompanied by one or more central hepatic vein injuries. The tip of the clamp passes easily through a large laceration of the left hepatic vein.

Figure 3. **Blood pressure curves for injured animals.** This graph contains composite mean arterial blood pressure (MAP) curves for the no fluid (NF), lactated Ringer's (LR) and Hextend® (HEX) groups. The liver injury is created at time zero. The drop in MAP just before injury is artificial secondary to liver manipulation for clamp placement. Fluid resuscitation is begun at time 30. Both LR and HEX resuscitation groups maintain similar MAP throughout resuscitation. The NF MAP remains significantly lower than the resuscitation groups.

Figure 4. **Blood loss, resuscitation and urine output volumes following injury.** Pre-resuscitation blood loss, lactated Ringer's (LR): 22 ± 5 mL/kg; Hextend® (HEX): 22 ± 8 mL/kg; NF: 18 ± 6mL/kg is similar in all groups ($P > 0.1$). Swine resuscitated with LR required 119 ± 78 ml/kg of fluid compared to 40 ± 21 mL/kg of fluid for the HEX swine ($P = 0.01$). LR (21 ± 13 mL/kg) animals produced significantly more urine compared to HEX (10 ± 4 mL/kg, $P = 0.03$) or no fluid (NF) (4 ± 2 mL/kg, $P < 0.01$) animals. HEX animals produced significantly more urine compared to NF animals ($P < 0.01$).

Figure 5. **Baseline and end of study hematocrit.** This graph shows the baseline and end of study (120 minutes) hematocrits for the three uncontrolled hemorrhagic shock groups: lactated Ringer's (LR), Hextend® (HEX), and no fluid (NF). At 120 minutes LR and HEX animals develop profound anemia compared to their baselines (*$P < 0.001$) and compared to the NF animals (**$P < 0.001$).

Figure 6. **Lung neutrophil sequestration.** Sequestered neutrophil data are presented as mean number of neutrophils per high-power field ± SEM. Sham animals had significantly more sequestered neutrophils than control animals, $P < 0.01$, and all uncontrolled hemorrhagic shock (UHS) animals had significantly more sequestered neutrophils than shams or controls, $P < 0.01$. There was no difference between the UHS groups, $P > 0.4$

Figure 1

Figure 2

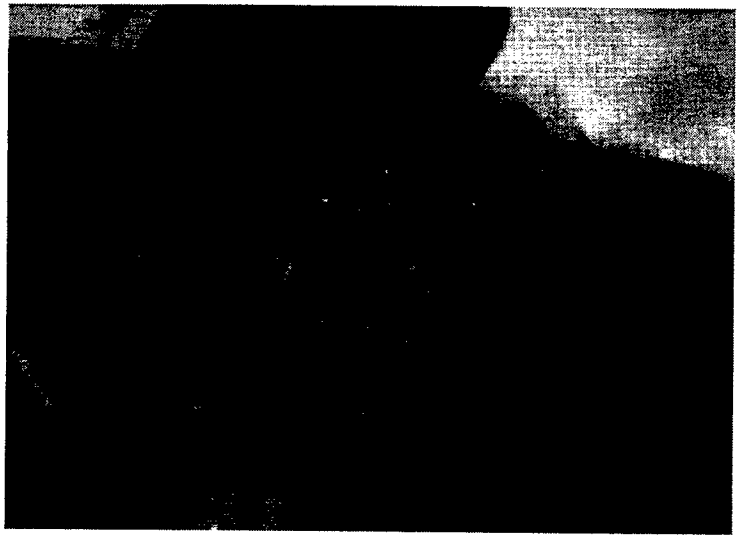


Figure 3

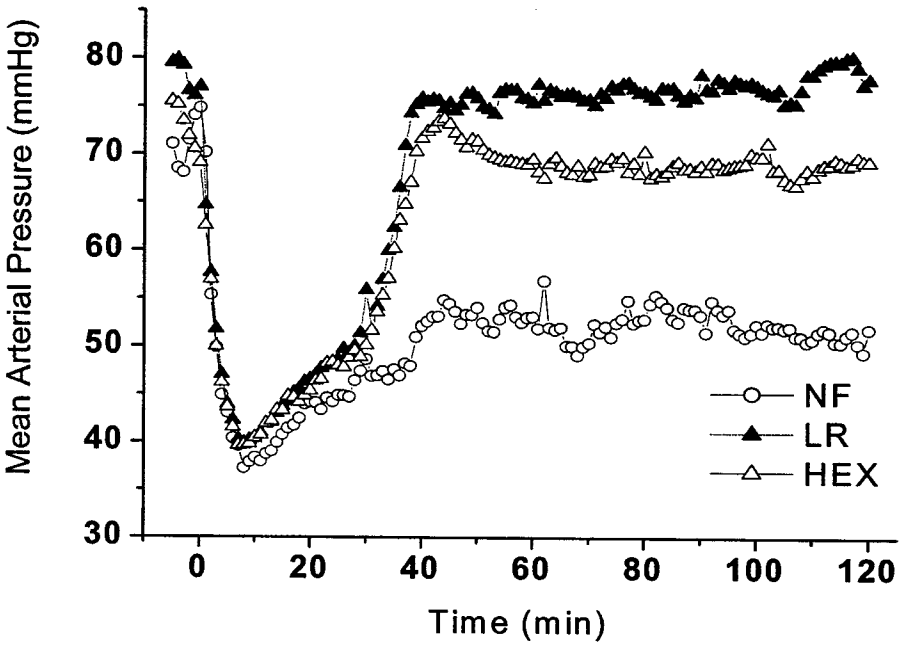
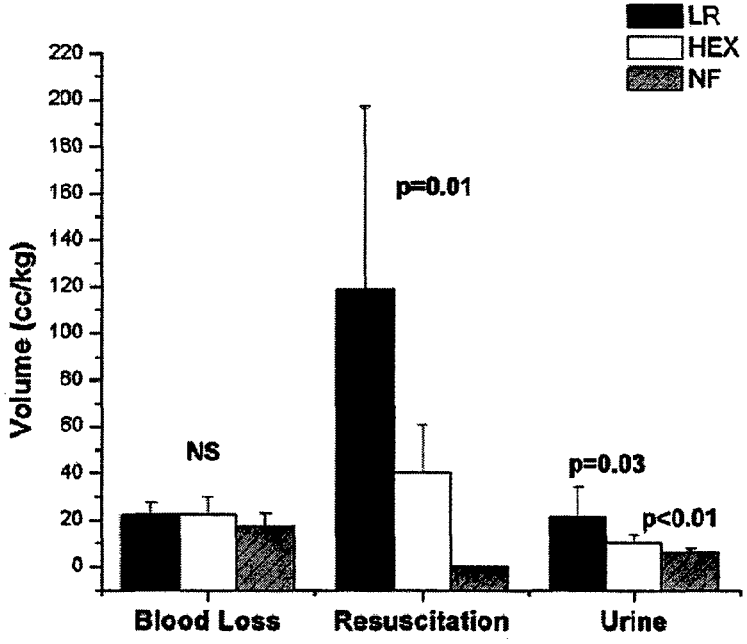


Figure 4



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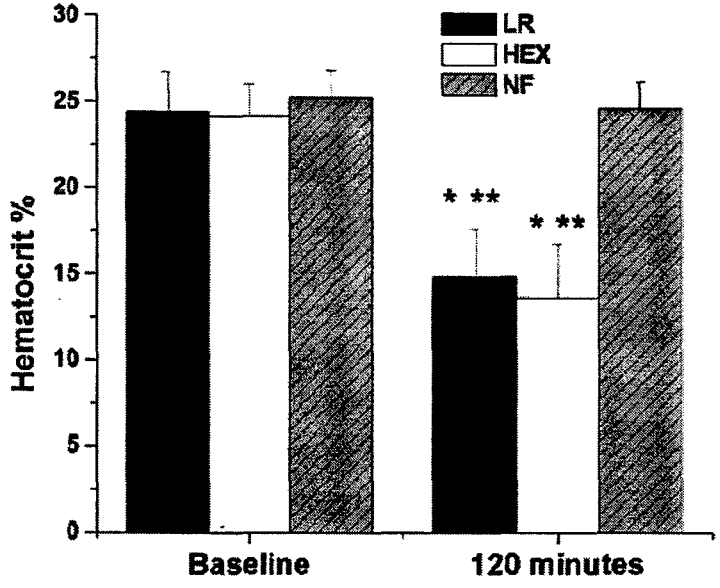

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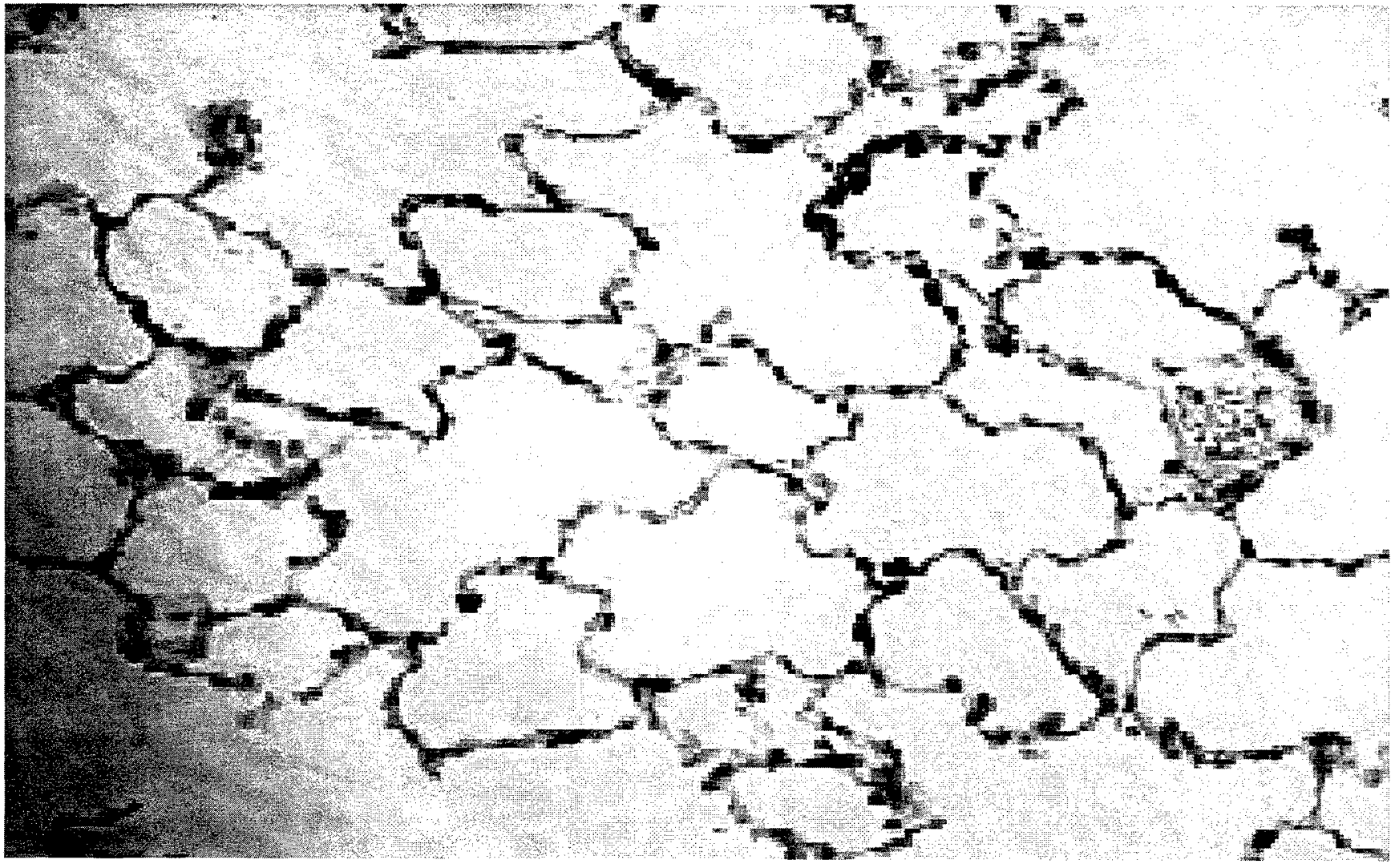

Table 1

Comparisons of Mean Weight, Mean Injury Temperature, and Mean Number of Vessels Injured between Treatment Groups.

	Lactated Ringer's	Hextend®	*p* Value
Mean Weight (kg)	35.5 +/- 3.1	34.4 +/- 2.4	NS
Mean Injury Temperature (°C)	37.6 +/- 0.7	37.7 +/- 0.8	NS
Mean Vessels Injured	1.1 +/- 0.3	1.4 +/- 0.5	NS

Table 2

	Control	Sham	NF	LR	HEX	p-value		
n	6	6	6	10	10	NF *v.* LR	NF *v.* HEX	LR *v.* HEX
PMNs	6.9 +/- 1.3	12 +/-2	22 +/- 3	21 +/-7	21 +/-6	0.426	0.353	0.972
IL-6	1 +/- 1.6	3 +/- 3	1.4 +/-1	15 +/-20	21 +/-19	0.057	0.009	0.508
G-CSF	1 +/- 1.0	2 +/- 1	4 +/-3	127 +/- 163	325 +/- 366	0.040	0.021	0.143
TNF-α	1 +/- 0.5	2 +/- 2	10 +/- 2	106 +/- 127	167 +/- 136	0.039	0.005	0.315

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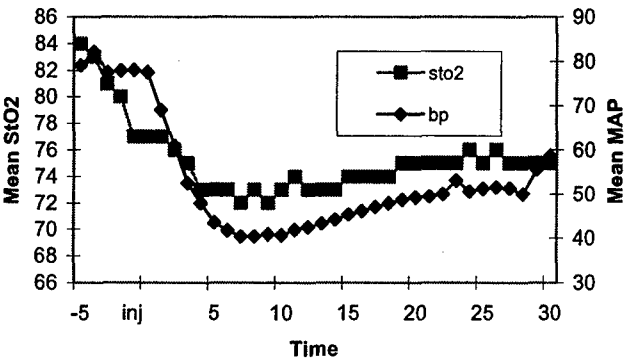
The Correlation Between Mean Arterial Blood Pressure and Tissue Oxygenation After Uncontrolled Hemorrhagic Shock

We hypothesized that spontaneous elevation in blood pressure following cessation of bleeding after uncontrolled hemorrhage is associated with improved tissue oxygenation. 25 anesthetized swine (35 ± 3 kg) underwent carotid line placement, celiotomy and splenectomy. Tissue oxygenation (StO2) was measured utilizing infrared spectroscopy. A Grade V liver injury was made and animals were allowed to bleed without resuscitation for 30 minutes. Means were compared using a paired t-test and correlations were measured using Pearson's ?. Mean blood loss was 642 +/- 108 ml. Changes in blood pressure and tissue oxygenation are shown in the figure. There was excellent overall correlation between MAP and StO2 ($r = 0.90$, $p<0.01$). Celiotomy was associated with a significant reduction in StO2 that was not reflected by the blood pressure (-5 minutes until injury, $p < 0.01$). The correlation between MAP and StO2 during the periods after injury and bleeding cessation and from bleeding cessation to the end of the study were significant ($r = 0.96$, $p <0.01$ and $r = 0.84$, $p < 0.01$).



Celiotomy results in diminished tissue oxygenation that is not reflected by blood pressure. There is excellent correlation between blood pressure and tissue oxygenation after hemorrhagic shock. Elevation in blood pressure following cessation of hemorrhage is associated with improved tissue oxygenation.

Introduction: The optimal fluid quantity and composition for the treatment of hemorrhagic shock is not known. An ideal fluid for early resuscitation would restore perfusion without increasing blood loss, hypothermia, acidosis, or coagulopathy. This study sought to examine the effects of a single bolus of hypertonic saline (HTS) with or without (±) dextran (D) following uncontrolled hemorrhagic shock (UHS) and to determine the optimal fluid composition.

Methods: 50 female Yorkshire crossbred swine were anesthetized and underwent invasive line placement, celiotomy, splenectomy, and suprapubic catheterization. Following a 15-minute stabilization period, a grade V liver injury was created. After 30 minutes of UHS, blinded resuscitation was initiated with a single 250cc fluid bolus. Animals were randomized to 5 groups: normal saline (NS), 3%HTS (3%), 3%HTS/6%D (3%/D), 7.5%HTS (7.5%), or 7.5%HTS/6%D (7.5%/D). Mean arterial pressure (MAP) and tissue oxygen saturation (StO2) were continuously monitored. Laboratory data were collected every 30 minutes. Animals were sacrificed 120 minutes after injury. ANOVA was used to compare groups. Significance was defined as $p<0.05$.

Results: Baseline weight (33kg), baseline MAP, number of central veins injured, nadir MAP, and nadir StO2 were similar in all groups. Two NS and two 3% animals did not survive. Fluids containing dextran produced a significantly greater increase in MAP ($p<0.02$). Animals receiving 3%/D maintained a higher MAP 90 minutes after fluid bolus. 7.5% ± D produced a significantly greater initial increase in StO2 ($p<0.05$). This effect declined within 15-30 min while 3%/D continued to improve tissue oxygenation throughout the study. Primary (23.3 ± 5.7cc/kg) and secondary (1.8 ± 0.9cc/kg) blood loss and resuscitation volumes (7.3 ± 0.7cc/kg) were equal in all groups. 7.5% ± D animals produced significantly more urine than any other group ($p<0.03$). Baseline laboratory values were similar in all groups. After resuscitation, significant differences developed between groups in hematocrit, fibrinogen, urine Na, serum Na, and serum Cl.

120 min values	NS	3%	3%/D	7.5%	7.5%/D
HCT	23.8 ± 3.0 bde	22.4 ± 2.7 bde	19.6 ± 2.1 abc	20.7 ± 2.2 ab	16.7 ±2 .7 f
Fibrinogen	161 ± 47 b	186 ± 54 b	150 ± 47	185 ± 51 b	127 ± 31 ce
Urine Na	144 ± 3 be	147 ± 3 be	146 ± 3 be	153 ± 5 acd	154 ± 3 acd
Serum Na	136 ± 2 f	139 ± 1 abe	140 ± 1 abe	147 ± 1 acd	148 ± 1 acd
Serum Cl	107 ± 3 bde	109 ± 4 be	111 ± 3 abe	118 ± 5 acd	120 ± 4 acd
$p<0.05$ a: *v* NS, b: *v* 7.5%/D, c: *v* 3%, d: *v* 3%/D, e: *v* 7.5%, f: *v* all others					

Conclusions: A single bolus of 3%/D following uncontrolled hemorrhagic shock produces an adequate and sustained rise in mean arterial pressure and tissue oxygen saturation. Resuscitation with 7.5% ± D produces significantly increased urine output. This may account for the decline in mean arterial pressure and tissue oxygen saturation in these groups over time. A single bolus of 7.5%/D results in significant dilutional anemia and hypofibrinogenemia.

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7.5% SALINE WITH DEXTRAN RESUSCITATION CAUSES DYSFUNCTIONAL INFLAMMATION IN UNCONTROLLED HEMORRHAGIC SHOCK

Introduction: Hypertonic saline solutions for resuscitation have shown benefit in both human and animal studies. The ideal composition of the fluid is unknown. We hypothesized the addition of dextran to 7.5% hypertonic saline would not affect expression of pro-inflammatory mediators after uncontrolled hemorrhagic shock.

Methods: 62 Yorkshire crossbred female swine were randomized into 7 cohorts: control, sham, 0.9% saline (NS), 7.5% saline (7.5S), and 7.5% saline with 6% dextran (7.5D). Controls underwent anesthesia and lung harvest. All other animals underwent invasive lines, laparotomy, and splenectomy. Shams underwent two hours of general anesthesia and lung harvest. Resuscitation swine received a grade V liver injury followed by 30 minutes of uncontrolled hemorrhage; one 250 mL bolus of blinded, randomized intravenous fluid was then given, and lung was harvested 90 minutes after resuscitation. Total RNA was extracted from lung tissue, transcribed into cDNA, and subjected to quantitative, real-time polymerized chain reactions to measure expression of GCSF, IL-6, and TNF-α. Fold-increases in gene expression for each lung sample was calculated by calibration with 18s rRNA expression and normalization to control swine. The Mann-Whitney test was used to compare fold-increases.

Results: Fold increases (±SD) are reported in the table.

Cohort	GCSF	IL-6	TNF-a
Control	1.00 ±2.72 [a]	1.00 ±1.32	1.00 ±0.95
Sham	1.39 ±3.65[a]	2.36 ±4.37	2.19 ±1.70
NS	1.80 ±4.24[b]	12.57 ±16.51	4.42 ±7.46
7.5S	0.66 ±1.50[a]	3.92 ±5.59	0.80 ±1.14
7.5D	2.50 ±5.55	30.17 ±58.10	2.11 ±3.89

[a] $p<0.05$ versus 7.5D, [b] $p<0.05$ versus 7.5S

Conclusions: Fluid resuscitation with 7.5D increases expression of GCSF; use of 7.5S alone does not produce the same response. The addition of dextran to 7.5% saline in fluid resuscitation results in potentially harmful, dysfunctional inflammation.